

WHAT IS CLAIMED IS:

1. A bispecific molecule comprising an antibody cross-linked to one or more antigen-binding antibody fragments via a chemical cross-linker, wherein said antibody binds a C3b-like receptor, and wherein each of said one or more antigen-binding antibody fragments binds an antigenic molecule.

2. The bispecific molecule of claim 1, wherein said one or more antigen-binding antibody fragments do not comprise an Fc domain.

3. The bispecific molecule of claim 1, wherein said one or more antigen-binding antibody fragments comprise an antigen-binding antibody fragment selected from the group consisting of an Fab, an Fab', an (Fab')<sub>2</sub>, and an Fv fragment of an immunoglobulin molecule.

4. The bispecific molecule of claim 1, wherein said one or more antigen-binding antibody fragments comprise a single-chain Fv fragment or a single-chain Fv fragment fused with a constant domain of an immunoglobulin molecule.

5. The bispecific molecule of claim 3, wherein at least one of said antigen-binding antibody fragments is a fusion protein further comprising a linker peptide fused to said Fab, Fab', (Fab')<sub>2</sub>, or Fv fragment, wherein said linker peptide is covalently bound to said chemical cross-linker.

6. The bispecific molecule of any one of claims 1-5, wherein at least one of said one or more antigen-binding antibody fragments is cross-linked at a predetermined site to said antibody that binds said C3b-like receptor.

7. The bispecific molecule of claim 6, wherein said predetermined site is a cysteine residue in said antigen-binding antibody fragment.

8. The bispecific molecule of claim 7, wherein said predetermined site is the C-terminus of said at least one antigen-binding antibody fragment.

9. The bispecific molecule of claim 1, wherein said antibody that binds a C3b-like receptor is a monoclonal antibody.

10. The bispecific molecule of claim 9, wherein said monoclonal antibody is a murine monoclonal antibody.

11. The bispecific molecule of claim 10, wherein said murine monoclonal antibody is 7G9.

12. The bispecific molecule of claim 9, wherein said monoclonal antibody is a humanized monoclonal antibody.

5 13. The bispecific molecule of claim 9, wherein said monoclonal antibody is a human monoclonal antibody.

14. The bispecific molecule of claim 9, wherein said one or more antigen-binding antibody fragments bind the protective antigen (PA) protein of *Bacillus anthracis* (Anthrax).

10 15. The bispecific molecule of claim 14, wherein said one or more antigen-binding antibody fragments are Fab fragments of murine monoclonal antibody 14B7.

16. The bispecific molecule of claim 14, wherein said one or more antigen-binding antibody fragments are single chain antibody fragments derived from murine monoclonal antibody 14B7.

15 17. The bispecific molecule of any one of claims 1-5, 15 and 16, wherein said bispecific molecule binds said antigenic molecule with an activity at least 5% of that the antibody from which said antigen-binding antibody fragment is derived.

18. The bispecific molecule of claim 17, wherein said bispecific molecule binds said antigenic molecule with an activity at least 15% of that the antibody from which said  
20 antigen-binding antibody fragment is derived.

19. The bispecific molecule of claim 18, wherein said bispecific molecule binds said antigenic molecule with an activity at least 25% of that of the antibody from which said antigen-binding antibody fragment is derived.

20. The bispecific molecule of claim 19, wherein said bispecific molecule binds  
25 said antigenic molecule with an activity at least 50% of that of the antibody from which said antigen-binding antibody fragment is derived.

21. The bispecific molecule of claim 20, wherein said bispecific molecule binds said antigenic molecule with an activity at least 90% of that of the antibody from which said antigen-binding antibody fragment is derived.

22. The bispecific molecule of claim 21, wherein said bispecific molecule binds said antigenic molecule with an activity at least 99% of that of the antibody from which said antigen-binding antibody fragment is derived.

23. The bispecific molecule of any one of claims 1-5, 15 and 16, wherein said  
5 bispecific molecule binds said antigenic molecule with an activity at least 5% of that of said antigen-binding antibody fragment not cross-linked with said antibody that binds said C3b-like receptor.

24. The bispecific molecule of claim 23, wherein said bispecific molecule binds said antigenic molecule with an activity at least 15% of that of said antigen-binding antibody  
10 fragment not cross-linked with said antibody that binds said C3b-like receptor.

25. The bispecific molecule of claim 24, wherein said bispecific molecule binds said antigenic molecule with an activity at least 25% of that of said antigen-binding antibody fragment not cross-linked with said antibody that binds said C3b-like receptor.

26. The bispecific molecule of claim 25, wherein said bispecific molecule binds  
15 said antigenic molecule with an activity at least 50% of that of said antigen-binding antibody fragment not cross-linked with said antibody that binds said C3b-like receptor.

27. The bispecific molecule of claim 26, wherein said bispecific molecule binds said antigenic molecule with an activity at least 90% of that of said antigen-binding antibody fragment not cross-linked with said antibody that binds said C3b-like receptor.

28. The bispecific molecule of claim 26, wherein said bispecific molecule binds  
20 said antigenic molecule with an activity at least 99% of that of said antigen-binding antibody fragment not cross-linked with said antibody that binds said C3b-like receptor.

29. A method of producing a bispecific molecule, comprising

(a) producing an antigen-binding antibody fragment comprising a cysteine residue  
25 by a host cell such that said cysteine residue in said antigen-binding antibody fragment is maintained as a free thiol;

(b) recovering said antigen-binding fragment having said free thiol; and

(c) contacting said antigen-binding antibody fragment having said free thiol with a derivatized antibody that binds a C3b-like receptor under appropriate conditions such that

said derivatized antibody cross-links to said antigen-binding antibody fragment at said free thiol;

thereby producing said bispecific molecule.

30. The method of claim 29, wherein said antigen-binding antibody fragment is  
5 secreted by said host cell.

31. The method of claim 29, wherein said derivatized antibody that binds a C3b-like receptor is derivatized with a maleimide.

32. The method of claim 31, wherein said maleimide is sulfosuccinimidyl  
4-(N-maleimidomethyl) cyclohexane-1-carboxylate.

10 33. The method of claim 31, wherein said maleimide is NHS-poly(ethylene glycol)-maleimide.

34. A method of producing a bispecific molecule, comprising cross-linking an antibody with an antigen-binding antibody fragment, wherein said antibody binds a C3b-like receptor and said antigen-binding antibody fragment binds an antigenic molecule.

15 35. A method of producing a bispecific molecule, comprising

(a) producing a thiol-derivatized antigen-binding antibody fragment such that said antigen-binding antibody fragment comprises a free thiol;

(b) producing a maleimide-derivatized antibody that binds a C3b-like receptor such that said antibody comprises a maleimide; and

20 (c) contacting said antigen-binding antibody fragment containing said free thiol with said antibody containing said maleimide under conditions such that said antibody and said antigen-binding antibody fragment cross-link via said maleimide and said free thiol;

thereby producing said bispecific molecule.

36. The method of claim 35, wherein said antigen-binding antibody fragment is  
25 derivatized with N-succinimidyl-S-acetyl-thioacetate (SATA).

37. The method of claim 36, wherein said antigen-binding antibody fragment is derivatized at a molar ratio of about 1:3 to about 1:6 antigen-binding antibody fragment:SATA.

38. The method of claim 35, wherein said antibody that binds a C3b-like receptor is derivatized with sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate.

39. The method of claim 35, wherein said antibody that binds a C3b-like receptor is derivatized with NHS-poly(ethylene glycol)-maleimide.

5           40. The method of any one of claims 35-39, wherein said step (c) is carried out by a method comprising mixing said thiol-derivatized antigen-binding antibody fragment and said maleimide-derivatized antibody that binds a C3b-like receptor at a molar ratio of about 1:1.

10           41. The method of any one of claims 35-39, wherein said step (c) is carried out by a method comprising mixing said thiol-derivatized antigen-binding antibody fragment and said maleimide-derivatized antibody that binds a C3b-like receptor at a molar ratio of about 2:1.

42. The product as produced by the method of any one of claims 34-39.

15           43. A polyclonal population of bispecific molecules comprising a plurality of different bispecific molecules, each bispecific molecule in said plurality comprising an antibody cross-linked via a chemical cross-linker to one or more antigen-binding antibody fragments, wherein said antibody binds a C3b-like receptor, and wherein said antigen-binding fragments bind an antigenic molecule.

20           44. The polyclonal population of bispecific molecules of claim 43, wherein said one or more antigen-binding antibody fragments do not comprise an Fc domain.

45. The polyclonal population of bispecific molecules of claim 43, wherein said one or more antigen-binding antibody fragments comprise an antigen-binding antibody fragment selected from the group consisting of an Fab, an Fab', an (Fab')<sub>2</sub>, and an Fv fragment of an immunoglobulin molecule.

25           46. The polyclonal population of bispecific molecules of claim 43, wherein said one or more antigen-binding antibody fragments comprise a single-chain Fv fragment or a single-chain Fv fragment fused with a constant domain of an immunoglobulin molecule.

47. The polyclonal population of bispecific molecules of claim 45, wherein at least one of said antigen-binding antibody fragments is a fusion protein further comprising a

linker peptide fused to said Fab, Fab', (Fab')<sub>2</sub>, or Fv fragment, wherein said linker peptide is covalently bound to said chemical cross-linker.

48. The bispecific molecule of any one of claims 1-5 and 9-16, wherein said antigenic molecule is a molecule desired to be removed from the circulation of a mammal.

5 49. The bispecific molecule of claim 48, wherein said mammal is a human, and wherein said antibody binds CR1.

50. The bispecific molecule of any one of claims 1-5 and 9-16, wherein said antigenic molecule is an antigen of a pathogen, and wherein said antibody binds CR1.

51. The bispecific molecule of claim 50, wherein said pathogen is a bacterium.

10 52. The bispecific molecule of claim 50, wherein said pathogen is a virus.

53. The bispecific molecule of claim any one of claims 1-5 and 9-16, wherein said antigenic molecule is a toxin.

54. A method of treating a mammal having an undesirable condition associated with the presence of an antigenic molecule in its circulation, comprising the step of  
15 administering to the mammal a therapeutically effective amount of a bispecific molecule comprising an antibody cross-linked to one or more antigen-binding antibody fragments via a chemical cross-linker, wherein said antibody binds a C3b-like receptor on a blood cell of said mammal, and wherein each of said one or more antigen-binding antibody fragments binds said antigenic molecule.

20 55. The method of claim 54, wherein said one or more antigen-binding antibody fragments do not comprise an Fc domain.

56. The method of claim 54, wherein said one or more antigen-binding antibody fragments comprise an antigen-binding antibody fragment selected from the group consisting of an Fab, an Fab', an (Fab')<sub>2</sub>, and an Fv fragment of an immunoglobulin  
25 molecule.

57. The method of claim 54, wherein said one or more antigen-binding antibody fragments comprise a single-chain Fv fragment or a single-chain Fv fragment fused with a constant domain of an immunoglobulin molecule.

58. The method of claim 54, wherein at least one of said antigen-binding antibody fragments is a fusion protein further comprising a linker peptide fused to said Fab, Fab', (Fab')<sub>2</sub>, or Fv fragment, wherein said linker peptide is covalently bound to said chemical cross-linker.

5 59. The method of claim 54, wherein said antibody that binds a C3b-like receptor is a monoclonal antibody.

60. The method of claim 59, wherein said monoclonal antibody is a murine monoclonal antibody.

61. The method of claim 60, wherein said murine monoclonal antibody is 7G9.

10 62. The method of claim 59, wherein said monoclonal antibody is a humanized monoclonal antibody.

63. The method of claim 59, wherein said monoclonal antibody is a human monoclonal antibody.

15 64. The method of claim 60, wherein said one or more antigen-binding antibody fragments bind the protective antigen (PA) protein of *Bacillus anthracis* (Anthrax).

65. The method of claim 60, wherein said one or more antigen-binding antibody fragments are Fab fragments of murine monoclonal antibody 14B7.

20 66. The method of claim 60, wherein said one or more antigen-binding antibody fragments are single chain antibody fragments derived from murine monoclonal antibody 14B7.

67. The method of any one of claims 54-66, wherein said mammal is a human, and wherein said antibody binds CR1.

68. The method of claim 67, wherein said antigenic molecule is an antigen of a pathogen.

25 69. The method of claim 68, wherein said pathogen is a bacterium.

70. The method of claim 68, wherein said pathogen is a virus.

71. The method of claim 67, wherein said antigenic molecule is a toxin.

72. The method of claim 54-59, 65 and 66, wherein said bispecific molecule binds said antigenic molecule with an activity at least 5% of that the antibody from which said antigen-binding antibody fragment is derived.

73. The method of claim 72, wherein said bispecific molecule binds said antigenic molecule with an activity at least 15% of that the antibody from which said antigen-binding antibody fragment is derived.

74. The method of claim 73, wherein said bispecific molecule binds said antigenic molecule with an activity at least 25% of that of the antibody from which said antigen-binding antibody fragment is derived.

75. The method of claim 74, wherein said bispecific molecule binds said antigenic molecule with an activity at least 50% of that of the antibody from which said antigen-binding antibody fragment is derived.

76. The method of claim 75, wherein said bispecific molecule binds said antigenic molecule with an activity at least 90% of that of the antibody from which said antigen-binding antibody fragment is derived.

77. The method of claim 76, wherein said bispecific molecule binds said antigenic molecule with an activity at least 99% of that of the antibody from which said antigen-binding antibody fragment is derived.

78. The method of any one of claims 54-59, 65 and 66, wherein said bispecific molecule binds said antigenic molecule with an activity at least 5% of that of said antigen-binding antibody fragment not cross-linked with said antibody that binds said C3b-like receptor.

79. The method of claim 78, wherein said bispecific molecule binds said antigenic molecule with an activity at least 15% of that of said antigen-binding antibody fragment not cross-linked with said antibody that binds said C3b-like receptor.

80. The method of claim 79, wherein said bispecific molecule binds said antigenic molecule with an activity at least 25% of that of said antigen-binding antibody fragment not cross-linked with said antibody that binds said C3b-like receptor.



81. The method of claim 80, wherein said bispecific molecule binds said antigenic molecule with an activity at least 50% of that of said antigen-binding antibody fragment not cross-linked with said antibody that binds said C3b-like receptor.

5 82. The method of claim 81, wherein said bispecific molecule binds said antigenic molecule with an activity at least 90% of that of said antigen-binding antibody fragment not cross-linked with said antibody that binds said C3b-like receptor.

83. The method of claim 82, wherein said bispecific molecule binds said antigenic molecule with an activity at least 99% of that of said antigen-binding antibody fragment not cross-linked with said antibody that binds said C3b-like receptor.

10 84. A pharmaceutical composition for treating a mammal having an undesirable condition associated with the presence of an antigenic molecule in its circulation, comprising a therapeutically effective amount of the bispecific molecule of any one of claims 1-5 and 9-16 and a pharmaceutically acceptable carrier.

85. A method of producing a bispecific molecule, comprising

15 (a) producing a maleimide-derivatized antigen-binding antibody fragment such that said antigen-binding antibody fragment comprises a maleimide;

(b) producing a thiol-derivatized antibody that binds a C3b-like receptor such that said antibody comprises a free thiol; and

20 (c) contacting said antigen-binding antibody fragment containing said maleimide with said antibody containing said free thiol under conditions such that said antibody and said antigen-binding antibody fragment cross-link via said maleimide and said free thiol, thereby producing said bispecific molecule.

25 86. The method of claim 85, wherein said antigen-binding antibody fragment is derivatized with sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sSMCC).

87. The method of claim 86, wherein said antigen-binding antibody fragment is derivatized at a molar ratio of about 1:5 antigen-binding antibody fragment:sSMCC.

88. The method of claim 85, wherein said antibody that binds a C3b-like receptor is derivatized with N-succinimidyl-S-acetyl-thioacetate (SATA).

89. The method of claim 87, wherein said antibody that binds a C3b-like receptor is derivatized with N-succinimidyl-S-acetyl-thioacetate (SATA) at a molar ratio of about 1:12 antibody:SATA.

90. The method of claim 89, wherein said step (c) is carried out by a method comprising mixing said maleimide-derivatized antigen-binding antibody fragment and said thiol-derivatized antibody that binds a C3b-like receptor at a molar ratio of about 3.75:1 maleimide-derivatized antigen-binding antibody fragment:thiol-derivatized antibody.

91. The bispecific molecule as produced by the method of any one of claims 85-90.

92. The bispecific molecule of claim 91, wherein said antigen-binding antibody fragment is 14B7scAb and said antibody that binds a C3b-like receptor is the murine monoclonal antibody 7G9, and wherein said bispecific molecule has two 14B7scAb cross-linked to a 7G9.

93. A method of treating or preventing Anthrax infection in an animal, comprising administering to said animal a therapeutically or prophylactically sufficient amount of the bispecific molecule of claim 91.